CLAIMS

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1. A method of acquiring data on the mass of a substance fixed on a substrate, comprising the steps of:

using a structure including a partial structure to be disconnected by light to fix the substance on the substrate;

irradiating the substance fixed on the substrate with light for inducing the disconnection of the partial structure to be disconnected by light; and

analyzing the mass spectrum of the substance which is brought in an unfixed state by disconnecting the partial structure by the irradiation of light.

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2. The method according to claim 1, wherein means of analyzing the mass spectrum is matrix assisted laser desorption ionization time-of-flight mass spectrometry (to be abbreviated as MALDI-TOF MS).

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3. The method according to claim 2, wherein light for inducing the disconnection of the partial structure to be disconnected by light is a laser beam used for the analysis by MALDI-TOF MS.

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4. The method according to claim 3, wherein the laser beam used for the analysis by MALDI-TOF MS is a

nitrogen laser beam.

5. The method according to claim 1, wherein the substance fixed on the substrate is nucleic acid.

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6. The method according to claim 1, wherein a structure containing nitrobenzene is selected as the partial structure to be disconnected by the irradiation of light.

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7. The method according to claim 6, wherein the structure containing nitrobenzene is constructed with a compound represented by the following formula I or II:

$$\begin{array}{c|c} & & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & &$$

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Formula I

$$Br \xrightarrow{NO_2} H CH_2 n \xrightarrow{0} C - 0 - N X$$

Formula II

(wherein n is 3 or 4, and X is H or SO_3Na).

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- 8. The method according to claim 7, wherein the substrate is a glass substrate having a primary amino group formed on the surface, a thiol (SH) group is bonded to the terminal of the substance, and the amino group and the thiol group are bonded together by a compound represented by the formula I or the formula II through a reaction between the amino group and the succinimide ester site of the compound and a reaction between the thiol group and the bromobenzyl site of the compound.
- 9. The method according to claim 8, wherein the formation of a primary amino group on the glass substrate is carried out by using a silane coupling agent having the primary amino group.
- 10. The method according to claim 7, wherein the substrate is a glass substrate having a sulfanil group formed on the surface, an amino group is bonded to the terminal of the substance, and the thiol group and the amino group are bonded together by a compound represented by the formula I or the formula II through a reaction between the thiol group and the bromobenzyl site of the compound and a reaction

 25 between the amino group and the succinimide ester

site of the compound.

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11. The method according to claim 10, wherein the formation of a thiol group on the glass substrate is carried out by using a silane coupling agent having the thiol group.

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12. The method according to claim 6, wherein the structure containing nitrobenzene is constructed with a compound represented by the following formula III:

$$\begin{array}{c|c} & & & & \\ & &$$

Formula III

(wherein DMTrO is a dimethoxytrityloxy group and CNEt is a 2-cyanoethyl group).

- 13. The method according to claim 2, wherein a substance (matrix substance) for assisting the desorption and ionization of the substance fixed on the substrate is applied to at least a region to be used for the mass spectrometry of the substrate.
- 14. The method according to claim 13, wherein the thickness of the coating film of the matrix substance is large enough and required for the desorption and ionization of the substance fixed on

the substrate.

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15. A method of acquiring data on the mass of a bio-related substance on each matrix of a biochip having a plurality of bio-related substances fixed on a substrate in a matrix form by a structure including a partial structure to be disconnected by light, the method comprising the steps of:

irradiating the bio-related substance on each

10 matrix fixed on the substrate with light for inducing the disconnection of the partial structure to be disconnected by light; and

analyzing the mass spectrum of the bio-related substance which is brought in an unfixed state by disconnecting the partial structure by the irradiation of light.

- 16. The biochip having a plurality of biorelated substances fixed on a substrate in a matrix

 20 form, wherein the bio-related substance is fixed by a partial structure to be disconnected by light.
- 17. The biochip according to claim 16, wherein the bio-related substance fixed on the substrate is nucleic acid.
 - 18. The biochip according to claim 17, wherein

the nucleic acid is DNA.

19. The biochip according to claim 17, wherein the nucleic acid is RNA.

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- 20. The biochip according to claim 17, wherein the nucleic acid is PNA (peptide nucleic acid).
- 21. The biochip according to claim 16, wherein the partial structure to be disconnected by the irradiation of light has a structure containing nitrobenzene.
- 22. The biochip according to claim 21, wherein the structure containing nitrobenzene is constructed with a compound represented by the following formula I or II:

$$\begin{array}{c|c} & & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & \\ & & & \\ & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ &$$

Formula I

Br
$$NO_{2}$$
 $N - (CH_{2}) n - C - 0 - N$

Formula II

(wherein n is 3 or 4, and X is H or SO₃Na).

23. The biochip according to claim 21, wherein the structure containing nitrobenzene is constructed with a compound represented by the following formula III:

10 Formula III

(wherein DMTrO is a dimethoxytrityloxy group and CNEt is a 2-cyanoethyl group).

- 24. A method of acquiring data on the mass of a bio-related substance on each matrix of a biochip having a plurality of bio-related substances fixed on a substrate in a matrix form and the mass of a substance which interacts with the bio-related substance, the method comprising the steps of:
- fixing the bio-related substance on each matrix

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on the substrate by a structure including a partial structure to be disconnected by light;

placing the substance which interacts with the bio-related substance on each matrix of the biochip under an interactive condition;

irradiating the bio-related substance fixed on the substrate with light for inducing the disconnection of the partial structure to be disconnected by light; and

analyzing the mass spectra of the bio-related substance which has been brought in an unfixed state by the irradiation of light and the substance which has interacted with the bio-related substance in an unfixed state at the same time by disconnecting the partial structure.

25. An MALDI-TOF MS apparatus comprising:

means of moving a biochip to which bio-related substances are fixed with a structure to be disconnected by light to a position for analysis from a predetermined position; and

means of sequentially analyzing substances on each matrix in a specified order based on information on the shape of the biochip and the arrangement of matrices on the biochip at the position for analysis and moving the substances on each matrix from the position for analysis to the predetermined position.

- 72 -26. A biochip having bio-related substances fixed on a substrate, wherein the bio-related substances are fixed on the substrate by a partial structure to be disconnected by light. 5 27. A method of determining a base sequence of nucleic acid, comprising the steps of: (1) fixing, to a substrate, nucleic acid (DNA) complementary to a part or an entire part of a base 10 sequence on a 3'-side from a site desired for analysis of a base sequence of nucleic acid (DNA) desired for analysis of the base sequence as a primer used for performing an enzymatic nucleic acid extension reaction, using the nucleic acid desired 15 for analysis of the base sequence as a template, in a structure containing a partial structure to be disconnected by light on a 5'-side from the complimentary base sequence in the primer; (2) annealing the nucleic acid desired for 20 analysis of the base sequence to the primer fixed to the substrate at the complementary base sequence portion to form a hybrid; (3) performing the enzymatic extension reaction using the nucleic acid desired for analysis of the 25 base sequence as a template, on the substrate where the hybrid is formed, in the presence of appropriate amounts of 4 kinds of 2'-deoxynucleotide triphosphate

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(dNTP: N is A; adenine, G; guanine, C; cytosine, T; thymine) required for the enzymatic nucleic acid extension reaction and the 4 kinds of 2',3'-dideoxynucleotide triphosphate (ddNTP) as a terminator for an extension reaction;

- (4) removing the template nucleic acid from the substrate where the extension reaction is effected;
- (5) irradiating a plurality of extension reaction products having different chain lengths including a primer portion fixed to the substrate in a structure containing a partial structure to be disconnected by light, with light for disconnecting the partial structure to be disconnected, analyzing a molecular weight of the extension product
 - disconnected by the irradiation with light by a
 MALDI-TOF MS method, and clarifying a base sequence
 of an extension portion of the extension product
 based on an increase in a molecular weight from a
 molecular weight of the primer in the extension
 product; and
 - (6) analyzing a part or an entire part of the base sequence desired for analysis of nucleic acid desired for analysis of the base sequence, based on the base sequence of the extension portion.

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28. The method according to claim 27, wherein in the process (5), the irradiation light is laser

light used for analysis of the MALDI-TOF MS method.

- 29. The method according to claim 27, wherein the laser light used for analysis of the MALDI-TOF MS method is nitrogen laser light with a wavelength of 337 nm.
- 30. The method according to claim 27, wherein in the process (5), a structure containing

 10 nitrobenzene is selected as the partial structure to be disconnected by the irradiation with light.
- 31. The method according to claim 27, wherein the structure containing nitrobenzene is structured

 15 using a compound represented by the following formula I or II:

$$\begin{array}{c|c} & & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & &$$

Formula I

Br
$$H - (CH_2) n - C - 0 - N$$

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- 75 - (where, n=3 or 4, X=H or SO_3Na).

- 32. The method according to claim 31, wherein the substrate is a glass substrate on the surface of which a primary amino group is formed, a sulfanil (SH) group is bonded to a 5'-terminal of the primer, and the amino group is bonded to the sulfanil group via a compound represented by the formula I or a compound represented by the formula II by a reaction between the amino group and a succiimidoester site of the compound and a reaction between the sulfanil group and a bromobenzyl site of the compound.
- 33. The method according to claim 32, wherein the primary amino group is formed on the glass substrate by using a silane coupling agent having a primary amino group.
- 34. The method according to claim 31, wherein the substrate is a glass substrate on the surface of which a sulfanil group is formed, an amino group is bonded to a 5'-terminal of the primer, and the amino group is bonded to the sulfanil group via a compound represented by the formula I or a compound
- 25 represented by the formula II by a reaction between the sulfanil group and bromobenzyl site of the compound and a reaction between the amino group and a

succiimidoester site of the compound.

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- 35. The method according to claim 34, wherein the sulfanil group is formed on the glass substrate by using a silane coupling agent having a sulfanil group.
- 36. The method according to claim 30, wherein the structure containing nitrobenzene is structured using a compound represented by the following formula III:

$$\begin{array}{c|c} & & & \\ & & \\ & & \\ DMTr0 & & \\ &$$

Formula III

(wherein DMTrO is a dimethoxytrityloxy group and CNEt is a 2-cyanoethyl group).

- 37. The method according to claim 27, wherein enzyme used for the extension reaction has heat resisting property.
- 38. The method according to claim 27, wherein the substrate to which the primer is fixed is in a form of a nucleic acid chip in which a plurality of

primer nucleic acids are placed in a matrix,

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in the process (3), a part or an entire part of the primer nucleic acid is subjected to an enzymatic nucleic acid extension reaction together with the template thereof on the nucleic acid chip, and

in the process (4), the matrix portion subjected to the extension reaction is analyzed by the MALDI-TOF MS method.